PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 032030woMe/sto	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/09437	International filing date *(day/month/year)* 26.08.2003	Priority date *(day/month/year)* 28.08.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant EVOTEC NEUROSCIENCES GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.03.2004	20.12.2004
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09437

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-29 as originally filed

Claims, Numbers

1-11 received on 15.10.2004 with letter of 14.10.2004

Drawings, Sheets

1/11-11/11 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1,5 (both in part) 3,4,6-8,9-11 (all in full)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 1,5 (both in part) 4,9-11 (all in full) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3,6-8 (all in full)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,5
	No:	Claims	2
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1,2,5
Industrial applicability (IA)	Yes:	Claims	1,2,5
	No:	Claims	-

2. Citations and explanations

   see separate sheet

III. Non-establishment of opinion (Continuation)

2. SUPPORT (ART. 6 PCT)

Article 6 PCT requires that the matter for which protection is sought be defined in the claims in a clear and concise manner and that the claims be supported by the description. A claim is considered not to be supported in the sense of Article 6 PCT if the description does not disclose sufficient technical information to allow a person skilled in the art, using his common general knowledge, to carry out the invention within the whole area that is claimed, without undue burden and without using inventive skills. It should be noted that such lack of technical support can also be objected under Art. 5 PCT, the objection being that the disclosure is insufficient to enable the skilled person to carry out the "invention" over the whole area claimed. The requirements of Articles 5 and 6 PCT are both designed to reflect the principle that the terms of a claim should be commensurate with, or be justified by the disclosure of the invention.

The underlying application describes the identification of the differential expression of foap-13 in post-mortem brain tissue derived from AD patients compared to non-AD control individuals. An up-regulation of foap-13 gene transcription in the temporal cortex compared to the frontal cortex of Alzheimer patients was detected which was not detectable in non-AD individuals (p. 22, second §).
The analysis of the differential expression of foap-13 is limited to post-mortem brain tissue collected from AD and non-AD individuals. No experimental data are given demonstrating any differential expression of foap-13 protein.

The molecular mechanisms underlying different neurodegenerative diseases can be of quite different nature so that a molecular mechanism only observed in AD cannot credibly be extrapolated to any other neurodegenerative disease. Claims 1,5,9 and 10 referring to any neurodegenerative disease are therefore not considered to be supported in the sense of Article 6 PCT.

Furthermore, claims 1,5,9 and 10 lack support for the following additional reasons:
Firstly, there is no technical teaching disclosed in the description supporting a method as claimed in claim 1 for prognosticating or determining whether a subject is at increased risk of developing neurodegenerative diseases, including Alzheimer's disease, comprising

determining a level and/or an activity of a foap-13 gene transcription/ translation product. The description only describes the differential expression of foap-13 transcripts in post-mortem brain tissue of patients which were already suffering from Alzheimer's disease.
Secondly, there is no technical teaching in the description nor the drawings which could provide credible support for claims 9,10 and claims 1,5 insofar as they relate to the determination of the differential expression of foap-13 translation products: Due to the fact that the differential expression of the transcription product of a gene does not necessarily lead to a differential expression of the translation product, the described differential expression of foap-13 transcription products cannot provide support for the methods claimed insofar as they refer to the detection of the differential expression of foap-13 translation products.
Thirdly, claim 1 refers to the diagnosis of AD by determining a level and/or activity of a transcription product of the foap-13 gene in any sample of a subject; the differential expression of foap-13 mRNA however was only analysed in specific brain tissue samples, namely in a sample from the frontal cortex and in a sample from the temporal cortex.

From what is said above, it follows that the subject matter of claim 11, namely the use of an antibody specifically immunoreactive with foap-13 for detecting a pathological state which relates to AD in a cell, as well cannot be regarded as sufficiently supported as required by Article 6 PCT.

Claim 4 relates to a recombinant non-human animal comprising a non-native gene sequence coding for a foap-13 protein or fragment thereof wherein said non-human animal exhibit a predisposition to developing symptoms of neurodegenerative diseases or related disorders. The technical teaching disclosed in the underlying application does however not support such recombinant non-human animal as required by Article 6 PCT.

The lack of support for claims 4,9-11 is such, that no meaningful opinion can be formed.

An opinion in regard to novelty and inventive step will therefore only be given for those parts of claims 1 and 5 which are considered to be supported by the description, namely methods for the diagnosis of AD in post-mortem brain tissue samples comprising

determining a differential expression of the foap-13 gene in AD brain tissue compared to non AD tissue wherein an up-regulation of foap-13 mRNA in temporal cortex compared to frontal cortex is indicating AD and an assay for screening for a modulator of AD comprising the testing of the level of a transcription product of a gene coding for foap-13.

V. Reasoned statement (Continuation)

1. CITATIONS

Reference is made to the following documents:

D1: WO0153312 (2001-07-26) & DATABASE GENESEQ [Online] EBI; HUMAN POLYPEPTIDE SEQ. ID NO. 1861 22 October 2001 (2001-10-22), XP002269445 Database accession no. AAM38716

D2: WO0112662 (2001-02-22) & DATABASE GENESEQ [Online] EBI; LAL ET AL.: "Human membrane associated protein MEMAP-12" XP002270793 Database accession no. AAB74706

D4: EP-A-1 188 839 (EVOTEC NEUROSCIENCES GMBH) 20 March 2002 (2002-03-20)

2. NOVELTY (Art. 33(2) PCT)

The technical content/features of the kit claimed in claim 2, e.g. antibodies specific to foap-13 or nucleic acids for detecting foap-13 expression, are considered to be already disclosed in D1 and D2:

D1 discloses a protein with Seq. ID 2 (D1: Seq. ID 1861). Seq. ID 2 represents the protein sequence of the foap-13 protein. D1 as well refers to antibodies specifically reacting with such protein (p. 39, l. 1-6) and nucleic acids for the detection of expression patterns (p. 38 l. 18-35 and Seq.ID 3647).

D2 discloses the MEMAP-12 protein as well as the cDNA coding for MEMAP-12. The

sequence of the MEMAP-12 protein is identical to Seq. ID 2. Polynucleotides encoding MEMAP are used to detect and quantify gene expression in biopsied tissues in which expression of MEMAP is correlated with disease (p. 49, l. 31-p. 50, l. 19). The MEMAP cDNAs are used for the generation of a cDNA expression array (p. 65, l. 23-33, p. 66, l. 15-p. 68, l. 10). The production and diagnostic use of MEMAP specific antibodies is disclosed (p. 71, l. 7-21 and p. 49, l. 13-21).

In the light of D1 and D2, the subject matter of claim 2 is not novel.

3. Inventive step (Art. 33(3) PCT)

D4 is considered closest prior art for those parts of claims 1 and 5 considered to be supported. D4 discloses the use of flotillin mRNA expression as a marker for AD. The flotillin mRNA is differentially expressed in post-mortem brain tissue of AD- patients compared to non-AD individuals wherein the up-regulation of flotillin mRNA in temporal cortex compared to frontal cortex of AD patients is indicating AD. A method for screening for a modulator of AD comprising testing the level of a transcription product of a gene coding for flotillin is disclosed as well.

The difference between the methods disclosed in D4 and the methods claimed in claims 1 and 5 is that the foap-13 mRNA expression is used as a marker for AD instead of the flotillin mRNA expression.

Due to the fact that the use of foap-13 mRNA expression as a marker for AD appear not to show any effects going beyond those described in regard to the use of flotillin mRNA expression as a marker for AD, the problem of the underlying application must be seen in the provision of methods as disclosed in D4 using an alternative mRNA which, like flotillin mRNA is differentially expressed in specific post-mortem brain tissue samples, i.e. in the temporal cortex compared to frontal cortex of AD patients compared to non-AD individuals for use as a marker for AD.

The solution is the use of the foap-13 mRNA expression as a marker.

A person skilled in the art trying to solve the problem posed would try to identify further

mRNAs being differentially expressed in specific post-mortem brain tissue samples, i.e. in the temporal cortex compared to the frontal cortex of AD-patients compared to non-AD individuals, for use as an alternative marker for AD. Due to the fact that a person skilled in the art is aware that differences in the mRNA expression observed in different tissues under different physiological conditions encompass many different mRNAs and due to the fact that the identification of mRNAs differentially expressed in the temporal cortex compared to frontal cortex in AD and non-AD individuals is already described in D4, a person skilled in the art would have tried with a reasonable expectation of success to identify further such differentially expressed mRNAs without using inventive skills in order to solve the problem posed. In the absence of any unexpected technical effect associated with the use of foap-13 mRNA expression as a marker for AD, the identification/provision of differentially expressed foap-13 mRNA and the use of the foap-13 mRNA expression as a marker for AD is considered not to involve an inventive step because the identification of foap-13 mRNA being differentially expressed is just one of several mRNAs the person skilled in the art would have expected to identify as being differentially expressed as flotillin mRNA.

Therefore, insofar as having been examined, the subject matter of claims 1 and 5 is not considered to involve an inventive step.

AMENDED CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:
determining a level and/or an activity of
(i) a transcription product of the foap-13 gene, and/or
(ii) a translation product of the foap-13 gene and/or
(iii) a fragment, or derivative, or variant of said transcription or translation product, in a sample obtained from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a diseaseby the steps of:
(i) detecting in a sample obtained from said subject a level, or an activity, or both said level and said activity of a transcription product and/or of a translation product of a gene coding for foap-13, and (ii) comparing said level or activity, or both said level and said activity of a transcription product and/or of a translation product of a gene coding for foap-13 to a reference value representing a known health status and/or to a reference value representing a known disease status, and said level, or activity, or both said level and said activity, of said transcription product and/or said translation product is varied compared to a reference value representing a known health status, and/or is similar or equal to a reference value representing a known disease status, said kit comprising:
a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for

foap-13 and (ii) reagents that selectively detect a translation product of a gene coding for foap-13.

3. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i) the foap-13 gene and/or

(ii) a transcription product of the foap-13 gene and/or

(iii) a translation product of the foap-13 gene, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii).

4. A recombinant, non-human animal comprising a non-native foap-13 gene sequence or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

5. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the foap-13 gene, and/or

(ii) a transcription product of the foap-13 gene, and/or

(iii) a translation product of the foap-13 gene, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

6. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the foap-13 gene, and/or

(ii) a transcription product of the foap-13 gene, and/or

(iii) a translation product of the foap-13 gene, and/or

(v) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) administering a test compound to a non-human test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched non-human control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which non-human animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the non-human test animal indicates that the test compound is a modulator of said diseases or disorders.

7. The method according to claim 6 wherein said non-human test animal and/or said non-human control animal is a recombinant non-human animal which expresses foap-13, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native foap-13 gene transcriptional control element.

8. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to foap-13 protein, or to a fragment, or derivative, or variant thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said foap-13 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers;

(ii) adding a detectable, in particular a fluorescently labelled compound or a plurality of detectable, in particular fluorescently labelled compounds to be screened for said binding to said plurality of containers;

(iii) incubating said foap-13 protein, or said fragment, or derivative, or variant thereof, and said detectable, in particular fluorescently labelled compound or fluorescently labelled compounds;

(iv) measuring amounts of preferably fluorescence associated with said foap-13 protein, or with said fragment, or derivative, or variant thereof; and

(v) determining the degree of binding by one or more of said compounds to said foap-13 protein, or said fragment, or derivative, or variant thereof.

9. Use of a protein molecule, said protein molecule being a translation product of the gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

10. Use of a protein molecule, said protein molecule being a translation product of the gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

11. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the gene coding for foap-13, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, for detecting the pathological state of a cell in a sample obtained from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell which relates to Alzheimer's disease.